



Effects of 1,25-dihydroxyvitamin D₃ in an ovalbumin-induced allergic rhinitis model

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ABSTRACT

IL-17-producing Th17 cells play an important role in allergic airway diseases, but their local expression and regulation in allergic rhinitis (AR) is not well understood. This study investigated the effects of 1,25-dihydroxyvitamin D₃ (1,25-(OH)₂D₃) on T-bet expression, Th1 cells, Th2 cells, Th17 cells and IL-33-positive epithelial cells in AR. C57BL/6 mice were intranasally sensitized and challenged with ovalbumin (OVA), and 1,25-(OH)₂D₃ was intraperitoneally injected into AR mice. Cytokine levels were measured with enzyme-linked immunosorbent assays, phenotypic analysis of Th1, Th2 and Th17 cells in the spleen was completed with flow cytometry, and the CD4⁺ IL-17⁺ cells in the Nasopharynx-associated lymphoid tissue (NALT) and IL-33-positive cells in nasal mucosa was evaluated with immunofluorescence microscopy. AR mice shown significantly increased Th2 and Th17 cell ratio in spleen, IL-17 level in serum, IL-5 and IL-13 levels in NALF but a lower number of IL-33-positive epithelial cells and Th1 response (Th1 and Tbet⁺ Th1 cell ratio in the spleen and serum IFN-γ level) than the control mice. 1,25-(OH)₂D₃ treatment significantly decreased the number of sneezing, nasal rubbing, OVA-sIgE and IL-17 in serum, IL-5 and IL-13 levels in NALF, Th17 cell ratio in the spleen and the histological of nasal mucosal but increased the number of IL-33-positive epithelial cells in AR mice. However, 1,25-(OH)₂D₃ treatment did not significantly influence IFN-γ level in serum, and Th1, Tbet⁺ Th1 and Th2 cell ratio in spleen.

Thus, 1,25-(OH)₂D₃ may exert anti-allergic effects by suppressing Th17 responses and local production of IL-5 and IL-13 cytokines.

1. Introduction

Allergic rhinitis (AR) affects 10%–20% of the population worldwide [1]. Th1/Th2 imbalance is believed to play a critical role in airway allergic diseases, but this mechanism alone cannot fully explain AR pathology. Recent studies have demonstrated that increased IL-17 levels and Th17 cell number in nasal mucosa and peripheral blood are associated with clinical severity in patients with AR [2,3]. In addition, exogenous anti-IL-17 reduced the symptom severity, serum IgE level and Th2 response in ovalbumin (OVA)-induced AR mice [4], and IL-17-deficient AR mice show significantly reduced sneezing, serum IgE levels and local eosinophil count compared with wild-type AR mice [5]. 1,25-Dihydroxyvitamin D₃ (1,25-(OH)₂D₃), the active metabolite of vitamin D, has many effects on innate and adaptive immunity [6]. Clinical studies have reported that 1,25-(OH)₂D₃ improves the glucocorticoid response in resistant asthma patients [7], possibly because 1,25-(OH)₂D₃ inhibits a Th17 response in asthma [8,9]. A recent study reported that despite the limited associations between vitamin D and

AR, the inclusion of foods containing vitamin D in maternal diets during pregnancy can have beneficial effects during childhood [10].

Nasopharynx-associated lymphoid tissue (NALT) is considered the equivalent of Waldeyer's ring in humans and is important for local immunity in the upper respiratory tract in AR [11]. Although IL-17 is critical for the development of ectopic lymphoid tissues [2], the local expression and regulation of IL-17-producing Th17 cells in NALT is not well understood.

In this study, we mainly investigated the Th17 responses in the local and systemic immune responses of an AR model and evaluated whether vitamin D alters the Th17 response and alleviates inflammation in an OVA-induced allergic mouse model.

2. Materials and methods

2.1. Animals

Specific pathogen-free (SPF) 6-week-old female C57BL/6 mice were

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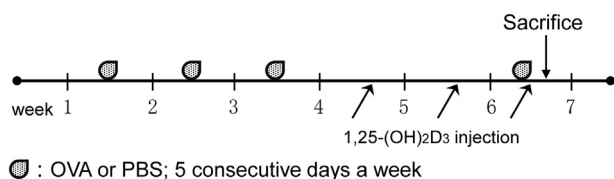


Fig. 1. The experimental protocol. AR mice were sensitized with 1% OVA (5 µl in each nostril) on weeks 1, 2 and 3. After two weeks of rest, mice were challenged with 1% OVA (5 µl in each nostril) for 5 days on week 6. AR mice were treated with subcutaneous injections of 1,25-(OH)₂D₃ (100 ng in 100 µl PBS) once every other day during weeks 4, 5 and 6. Control mice were sensitized, challenged and injected with PBS.

purchased from Hunan SJA Laboratory Animal Co., Ltd. and housed in the Guangxi Medical University Laboratory Animal Centre under SPF conditions. All experimental procedures were performed under approval from the Animal Care and Use Committee of Guangxi Medical University.

2.2. Induction of allergic rhinitis in mice

Mice were sensitized and challenged by intranasal administration of OVA as described by Virginia McMillan Carr [3]. Mice were divided into 3 groups and nine mice per group: (1) the negative control group, which was treated with PBS, (2) the AR group, which was treated with OVA, and (3) the AR with intraperitoneal 1,25-dihydroxyvitamin D₃ treatment (AR + 1,25-(OH)₂D₃) group. The procedure is shown in Fig. 1. Briefly, mice were sensitized by intranasal administration of 5 µl filter-sterilized 1.0% ovalbumin (OVA; Grade V; Sigma) in each side of the nares for 3 weeks (5 consecutive days per week, Monday to Friday). After two weeks of rest, the mice were challenged with OVA for another 5 days; the AR + 1,25-(OH)₂D₃ group was treated with subcutaneous injection of 100 ng 1,25-(OH)₂D₃ (MedChem Express, USA) in 100 µl PBS once every other day during weeks 4, 5 and 6, while the control and AR groups were injected with PBS instead of 1,25-(OH)₂D₃. Twenty-four hours after the final challenge and injection, mice were sacrificed.

2.3. Evaluation of nasal symptoms

To determine the change in nasal symptoms, the number of instances of sneezing and rubbing was counted for 15 min after the final challenge, by three observers blinded to treatment and compared with that of the AR group.

2.4. Isolation of NALT

As shown in Fig. 5C, the paired NALT was present on the nasal surface of the palate. The palate can be easily removed from the oral cavity, and NALT was clearly visible on the separated palate. NALT was collected from each group for immunofluorescence staining or flow cytometric analysis after isolation of the cells using a 70 µm cell strainer.

2.5. Histological assessment

Twenty-four hours after the final challenge and injection, mice were sacrificed with a lethal dose (120 mg/kg) of pentobarbital sodium. The skin, muscle and eyes were cleaned from the heads; then, the decapitated heads were fixed in 4% paraformaldehyde in PBS for 24 h and decalcified for 6 days in 10% EDTA. After decalcification, the noses were soft enough to cut with a razor blade, and the anterior portion of the nose and brain were excised, while the remainder of the nose was divided into two blocks and embedded in paraffin. Sections were stained with hematoxylin and eosin (H&E) to evaluate the histological changes and Periodic Acid-Schiff (PAS) to count the goblet cells.

The histological score was determined using a method modified from a previous report [4]. Briefly, the tissue was scored as follows: 0: no evidence of inflammatory cells, desquamation of cilia and glandular hypotrophy, 1: mild, occasional scattered inflammatory cells and desquamation of cilia, 2: moderate infiltration of inflammatory cells, desquamation of cilia and glandular hypotrophy, 3: severe, diffuse infiltration of inflammatory cells with desquamation of cilia and glandular hypotrophy. Sections were examined blindly with three different mucosal areas in each of two sections per mouse and six mice per group.

2.6. Immunofluorescence microscopy

Frozen NALT sections were blocked with 5% goat serum for 30 min and stained with rat anti-mouse CD4 (Abcam, USA; 1:150 dilution) and rabbit anti-mouse IL-17 (Bioss, China; 1:100 dilution) at 4 °C overnight before being incubated with Alexa Fluor 488-labeled goat anti-rat (Abcam; 1:500 dilution) and Alexa Fluor 594-labeled goat anti-rabbit (Abcam; 1:1000 dilution) secondary antibodies for 45 min at 37 °C. Nuclei were stained with DAPI (Bioss, China). Rat IgG (Bioss, China) and rabbit IgG (Bioss, China) were used for control staining.

Paraffin-embedded nasal tissues were cut into sections (5 µm), bathed in boiling citrate buffer (0.01 M, pH 6.0) for 30 min, washed in PBS 5 times and blocked with 5% goat serum for 30 min. Then, the slides were stained with rabbit anti-mouse IL-33 (Bioss, China) at 4 °C overnight and incubated with Alexa Fluor 594-labeled goat anti-rabbit secondary antibody (1:500 dilution) for 45 min at 37 °C, while nuclei were stained with DAPI. Rabbit IgG (Bioss, China) was used for control staining. The total number of IL-33-positive epithelial cells in two sections per mouse and six mice per group was counted. Sections were viewed with a fluorescence microscope (Olympus) and analyzed using Image-Pro Plus 6.0.

2.7. Flow cytometric analysis

Spleens and NALTs were separated into single cells using 70 µm cell strainers and cultured in RPMI 1640 with 10% FBS at a concentration of 1.0×10^6 cells/ml. Then, the cells were stimulated with 75 ng/ml PMA (Sigma-Aldrich), 1000 ng/ml ionomycin (Sigma-Aldrich) and 1 µl/ml BD Golgi Plug™ for 4 h. Cells were washed and resuspended in 100 µl of staining buffer (3% FBS in PBS), 1 µl/ml anti-CD16/32(2.4G2) was added to block Fcγ receptors, and surfacemarkers were stained with PerCP-Cy5.5-conjugated anti-mouse CD4(RM4-5) and PE-CF594-conjugated anti-mouse CD3(145-2C11). After the cells were washed and permeabilized (eBioscience, USA), they were stained intracellularly with PE-conjugated anti-mouse T-bet(O4-46), APC-conjugated anti-mouse IFN-γ(XMG1.2), PE-Cy7-conjugated anti mouse IL-4(11B11) and Alexa Fluor 488-conjugated anti-mouse IL-17(TC11-18H10). Then, the samples were washed and resuspended in staining buffer and analyzed with a FACSCalibur flow cytometer (BD FACSCanto II). The data were analyzed with FlowJo 10.0.7 (Tree Star).

2.8. Enzyme-linked immunosorbent assay

Blood samples were collected from the inner canthus vein and centrifuged to isolate serum, which was stored at −80 °C. The serum OVA-sIgE (Biolegend, USA), IFN-γ (CUSABIO, China) and IL-17 (Neobioscience, China) levels and nasal lavage fluid IL-5 and IL-13 (CUSABIO, China) levels were determined by ELISA, which was performed according to the manufacturer's instructions.

2.9. Statistical analysis

All statistical analyses were performed using GraphPad Prim 6.0. Data were expressed as the mean ± standard deviation (SD). Differences in parametric data were evaluated by using one-way

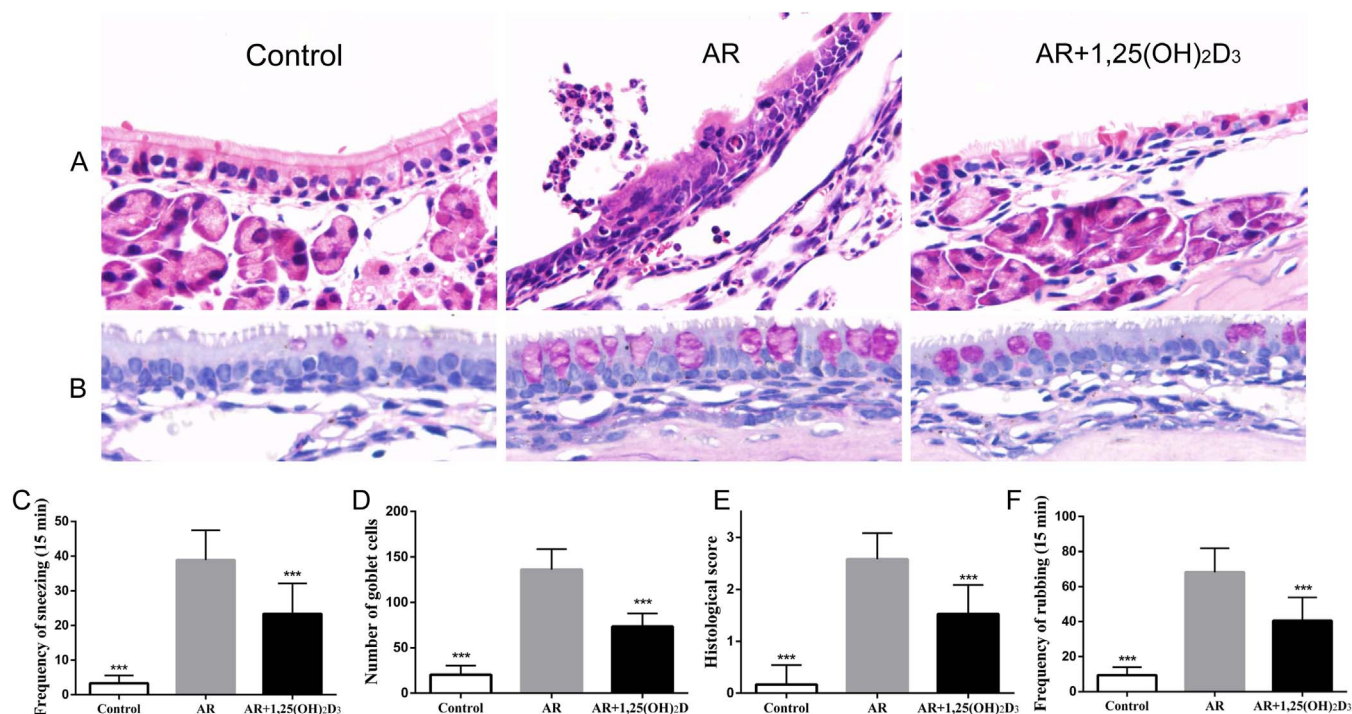


Fig. 2. Effects of 1,25-(OH)₂D₃ on histological changes and nasal symptoms in the OVA-induced AR model. Staining of (A) and (B), non-treated allergic rhinitis shows inflammatory cell infiltration into the nasal mucosa, goblet cell hyperplasia, desquamation of cilia and glandular hypotrophy, treatment with 1,25-(OH)₂D₃ significantly decreased histological changes. (A) shows H & E staining at $\times 400$ magnification, (B) shows PAS staining at $\times 400$ magnification. (C) and (D), the total number of sneezing and nasal rubbing motions was significantly reduced by 1,25-(OH)₂D₃, $n = 9$. (E). The number of goblet cells, $n = 6$. (F). Nasal histological scores, $n = 6$; Values represent the mean $\pm$ SD, *** $P < 0.001$ vs. the AR group.

analysis of variance (ANOVA), followed by Tukey's post-hoc test.

3. Results

3.1. 1,25-(OH)₂D₃ treatment alleviates histological changes, goblet cell hyperplasia and nasal symptoms in OVA-induced AR mice

AR mice showed rapid onset of sneezing and rubbing after OVA challenge, increased the number of goblet cells, desquamation of cilia, glandular hypotrophy and inflammatory cell infiltration was found in the mucosal area. Treatment with 1,25-(OH)₂D₃ significantly decrease the frequency of sneezing and rubbing, and the histological score, the number of goblet cells was lower in AR + 1,25-(OH)₂D₃ mice than AR mice (Fig. 2). These results indicate that 1,25-(OH)₂D₃ treatment reduced nasal symptoms and histological changes.

3.2. The Th17 cell percentage in spleen is decreased after 1,25-(OH)₂D₃ treatment in AR mice

The percentage of Th2 and Th17 cells in spleen was markedly higher in the AR mice than in the PBS-treated mice, and the mice treated with 1,25-(OH)₂D₃ showed a significantly smaller proportion of Th17 cells but not Th2 cells than the AR mice (Fig. 3F, Fig. 3G). T-bet has been reported to regulate the Th1/Th17 [5] and Th1/Th2 balance [12]. To determine whether the change in Th2 and Th17 responses were associated with T-bet, we analyzed T-bet expression in Th1 cells. The percentage of IFN- γ ⁺ Th1 and T-bet⁺ IFN- γ ⁺ Th1 cells was significantly lower in the AR mice than the control mice, but there was no significant difference in the percentage of IFN- γ ⁺ Th1, T-bet⁺ IFN- γ ⁺ Th1 cells in AR mice and AR + 1,25-(OH)₂D₃ mice (Fig. 3E, Fig. 3F). The above data indicate that while OVA-induced AR mice mainly showed downregulation of T-bet in Th1 cells and a corresponding increase in Th17 cells, treatment with 1,25-(OH)₂D₃ did not change T-bet expression, suggesting that 1,25-(OH)₂D₃ can directly suppress Th17 response independently of T-bet.

3.3. 1,25-(OH)₂D₃ suppresses IL-5, IL-13, IL-17 and OVA-sIgE production but not IFN- γ production in AR mice

While it is well known that AR is characterized by downregulation of the Th1-related cytokine IFN- γ and upregulation of Th2 associated cytokine. However, Th17-related IL-17 production in AR is not well understood. To study the mechanisms of 1,25-(OH)₂D₃ in AR, we analyzed the IL-17, IFN- γ and OVA-sIgE levels in serum, and the IL-5, IL-13 levels in NALF. The results indicated that the IFN- γ level in the AR mice was significantly downregulated relative to that in the control mice, but the IL-5, IL-13 and IL-17 level in serum was greatly upregulated in intranasal OVA-induced AR mice. While 1,25-(OH)₂D₃ treatment did not influence the IFN- γ level in the AR mice, it did decrease the IL-5, IL-13, IL-17 and OVA-sIgE levels significantly (Fig. 4). Our findings indicate that 1,25-(OH)₂D₃ inhibits IL-5 and IL-13 production in local and Th17 related-IL-17 production in system.

3.4. 1,25-(OH)₂D₃ suppresses Th17 cells in NALT

The distribution and expression of Th17 cells in NALT among three groups were detected by immunofluorescence staining of CD4 and IL-17 double positive cells (Fig. 5A), and NALTs in each group were combined to dissociate into single cells, and stained with CD3, CD4, IL-17 to analyze the percentage of Th17 cells by flow cytometry (Fig. 5D). We confirmed the expression of Th17 cells in NALT and 1,25-(OH)₂D₃ treatment decreased it than the AR mice (Fig. 5A). These findings suggest that Th17 cells may also be involved in nasal mucosal allergic reaction, and that treatment with 1,25-(OH)₂D₃ suppressed the expression of Th17 cells in NALT.

3.5. 1,25-(OH)₂D₃ shifts the balance toward IL-33-positive cells in OVA-induced AR mice

We investigated whether IL-33 is involved in AR through immunofluorescence staining of IL-33-positive cells and found that IL-33 is

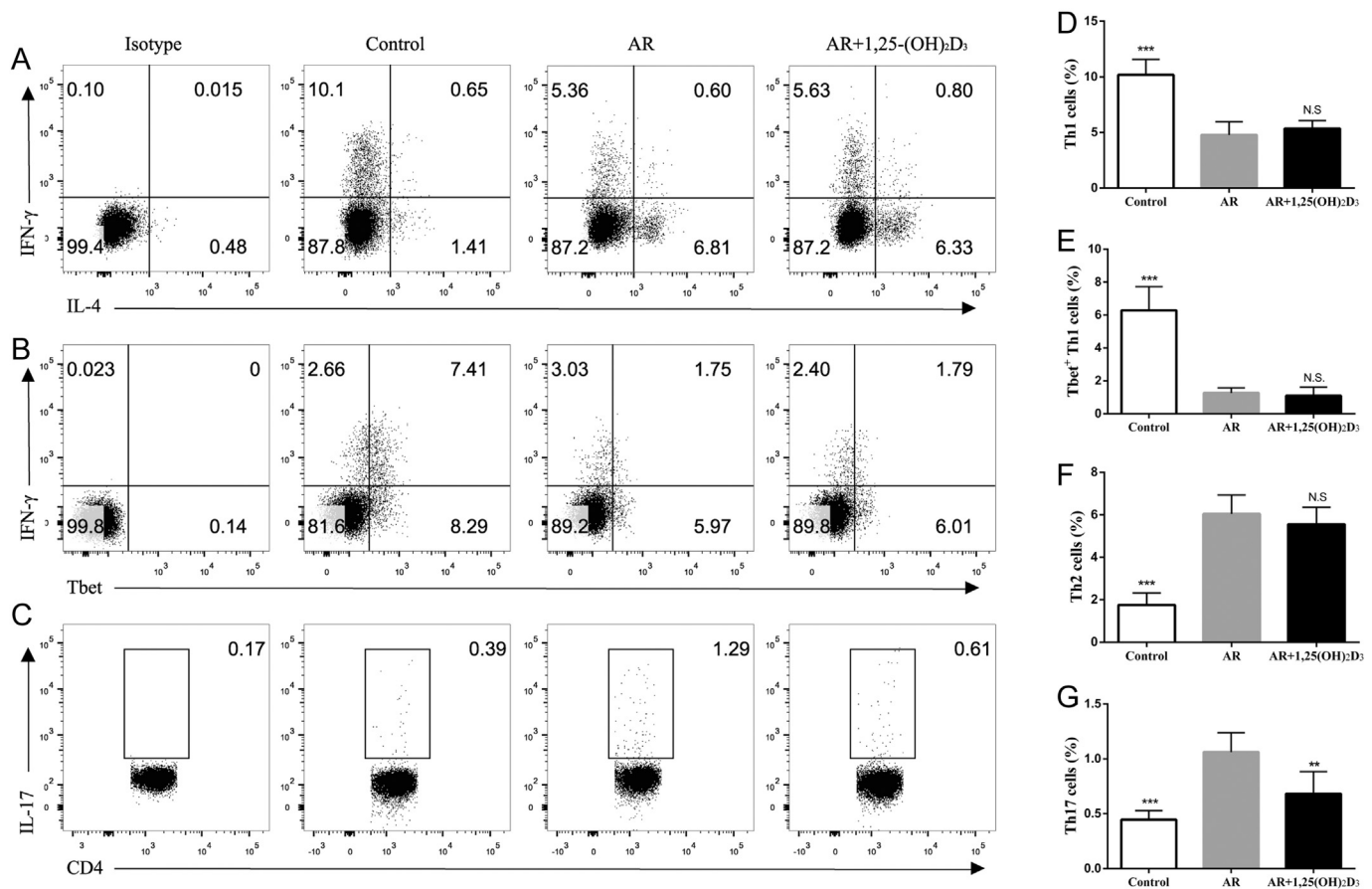


Fig. 3. (A), (B) and (C), In the CD4⁺ population, the proportion of Th17 cells, but not IFN-γ⁺ Th1, T-bet⁺ IFN-γ⁺ Th1, and Th2 cells in the spleen decreased after treatment with 1,25-(OH)₂D₃. Splenocytes were incubated with PMA and ionomycin in the presence of GolgiPlug™ mixture for 4 h. Cells were then stained with anti-CD3-PE-CF594 and anti-CD4-PerCP-Cy5.5 and further stained intracellularly with anti-IL-17-Alexa Fluor 488, anti-IFN-γ-APC, anti-IL-4-PE-Cy7, and anti-T-bet-PE to analyze the percentage of Th1, Th2 and Th17 cells by flow cytometry. (D) The frequency of IFN-γ⁺ Th1 cells in the spleen. (E) The frequency of T-bet⁺ IFN-γ⁺ Th1 cells in the spleen. (F) The frequency of Th2 cells in the spleen. (G) The frequency of Th17 cells in the spleen. Data are shown as the mean ± SD, *n* = 6. ***P* < 0.01, ****P* < 0.001, N.D.: not detected, and N.S.: no significance in comparison with the AR group.

mainly expressed in the nucleus of epithelial cells (Fig. 6A). Surprisingly, while IL-33 was highly expressed in the epithelium, intranasal instillation of OVA significantly reduced the number of IL-33-positive cells in AR mice, and 1,25-(OH)₂D₃ treatment increased this number (Fig. 6B). These findings suggest that IL-33 release from epithelial cells may be linked to allergen inflammation in AR mice and that treatment with 1,25-(OH)₂D₃ is advantageous in maintaining IL-33 at a steady state.

4. Discussion

Intranasal instillation of OVA has been shown to induce a stronger IL-17 response than IP injections of OVA with alum [13], and the addition of aluminum hydroxide can inhibit the secretion of Th1-polarizing cytokines [14]. Thus, to better investigate the Th17 and Th1 responses and more closely mimic human exposure history, we sensitized and challenged the mice in our study by intranasally administering a small volume of OVA. The percentage of Th17 cells in the spleen and the IL-17 level in the serum were higher in the AR mice than the control mice. However, even without the use of aluminum hydroxide, the AR mice demonstrated a significantly reduced Th1 response compared with the control mice.

To investigate the influence of 1,25-(OH)₂D₃ in AR, OVA-induced AR mice received subcutaneous injections of 100 ng 1,25-(OH)₂D₃ every other day, which is thought to be an optimal supplement of vitamin D in mice [15]. 1,25-(OH)₂D₃ administration significantly decreased the serum IL-17 level and the percentage of Th17 cells in

the spleen. As T-bet has a pivotal role in the development of IFN-γ-producing cells and suppresses the Th17 response by blocking the Th17 cell-specific transcription factor RORγt [16], we analyzed the T-bet⁺ IFN-γ⁺ Th1 cells in the spleen to determine whether the reduced Th17 response was regulated by T-bet. Treatment with 1,25-(OH)₂D₃ did not change the serum IFN-γ level or the percentage of T-bet⁺ IFN-γ⁺ Th1 cell in the spleen. These results suggested that 1,25-(OH)₂D₃ has a suppressive effect on IL-17-producing Th17 cells independent of T-bet-controlled Th1 responses and were in line with an in vitro study showing that 1,25-(OH)₂D₃ can directly inhibit the expression of RORα, RORγt, IL-17A, IL-17F, and IL-23R and reduce the frequency of IL-17⁺ cells [17].

With this study, we are the first to report the local presence of Th17 cells in NALT, and we found that NALT was covered with epithelial cells and exhibited a high percentage of Th17 cells. Other experiments have reported that APCs, DCs and B cells also reside in the NALT [18–20], and a NALT-deficient mouse study showed that these cells play a critical role in IgE production by local B cells [11]. Considering the unique role of NALT in the upper airway, we speculate that NALT may also be involved in local AR, which is characterized by local, but not systemic, IgE expression [21–24]. Despite the influence of IL-17 in recruiting eosinophils and neutrophils [25], it may also contribute to the development of inducible bronchus-associated lymphoid tissue [2] and enhance B-cell infiltration in human asthmatic airways [26]. In this study, we found that OVA-induced AR mice show a higher percentage of Th17 cells in NALT than PBS-treated mice, and treatment with 1,25-(OH)₂D₃ decreased the percentage of Th17 cells relative to

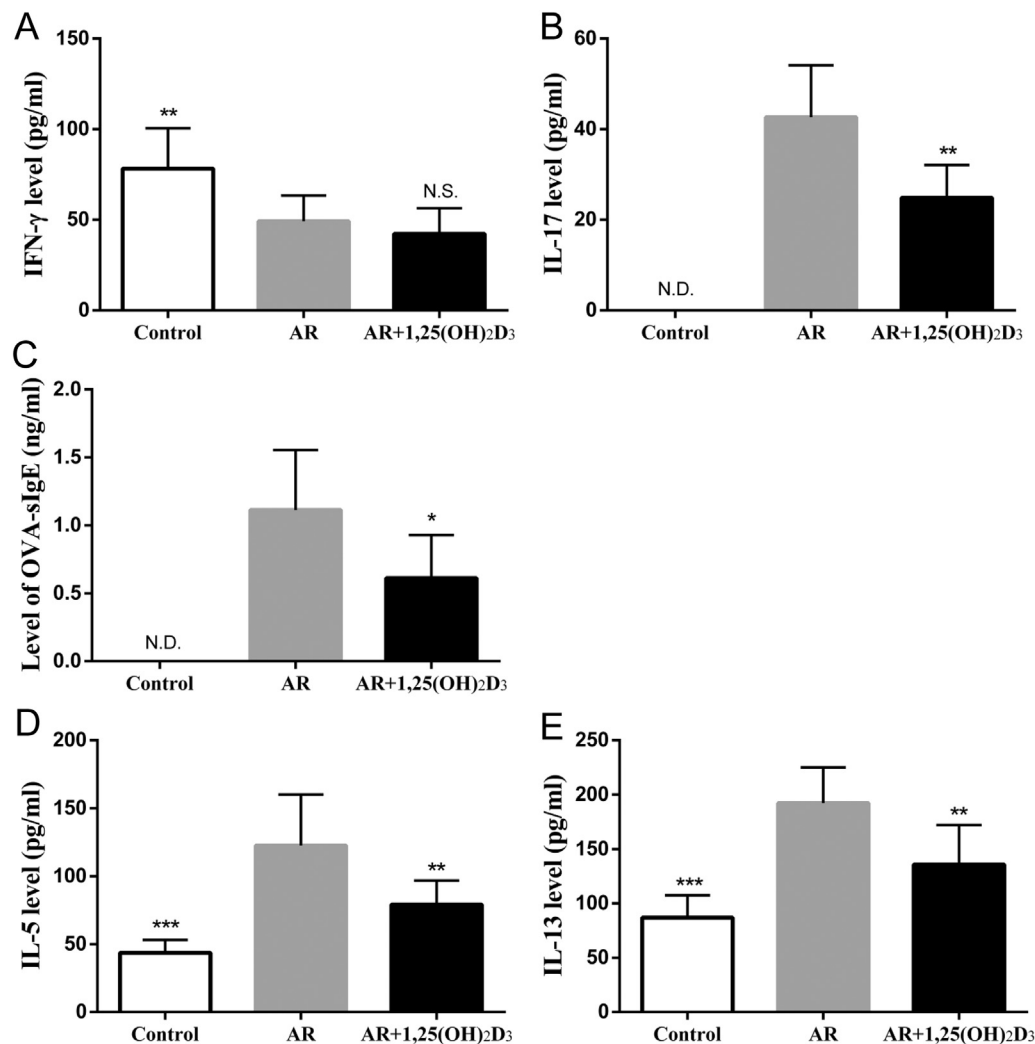


Fig. 4. Treatment with 1,25-(OH)₂D₃ reduced IL-17 and OVA-sIgE levels in serum, IL-5 and IL-13 levels in nasal lavage fluid. The serum levels of IFN-γ (A), IL-17 (B) and OVA-sIgE (C), the nasal lavage fluid levels of IL-5 (D) and IL-13 (E) were determined by ELISA. Values represent the mean ± SD, n = 9. *P < 0.05, **P < 0.01, ***P < 0.001, N.D.: not detected, in comparison with the AR group.

that in AR mice. Therefore, targeting IL-17-producing Th17 cells may aid in controlling AR in a local and systemic manner.

Although many reports have investigated the role of vitamin D in inhibiting the Th17 immune response [27–29], the effect of vitamin D on Th1 and Th2 responses remains controversial. In vitro studies, treatment of isolated splenocytes with 1,25-(OH)₂D₃ and OVA had two effects on the Th2 response: the IL-4 and IL-13 levels increased significantly, but the IL-5 and IFN-γ levels decreased [15]; Another study cultured isolated human PBMCs with dexamethasone plus or minus 1,25-(OH)₂D₃, showing that 1,25-(OH)₂D₃ has additive effects on Th1 inhibition but not Th2 cytokine production [30]. 1,25-Dihydroxyvitamin D₃ has reported to inhibit IL-4 levels through VDR but not GATA3 under polarizing conditions [31], and to enhance IL-4 levels as well as GATA3 under nonpolarizing conditions [32], this different may due to the cytokine environment [31]. And it has been reported that treating isolated murine CD4⁺ cells with 1,25-(OH)₂D₃ under Th1-polarizing conditions does not influence the frequency of IFN-γ + cells [17]. In vivo studies, VDR knockout mice develop a robust expression of IL-5 and IL-13 in BALF as well as elevated total IgE and OVA-sIgE in serum [33]. In addition, 1,25-(OH)₂D₃ can repress overt IgE-mediated mast cell activation in vitro and in vivo [34]. In our study, 1,25-(OH)₂D₃ suppressed the expression of IL-5 and IL-13 cytokines in NALF, but did not change the Th2 cell ratio in the spleen, suggesting that more than Th2 cells may be involved in mucosal allergic reactions, as it has

been reported that group 2 innate lymphoid cells (ILC2) are the major producer of IL-5 and IL-13 in murine lung [35], and IL-33-responsive innate lymphoid cells are an important source of IL-13 in nasal polyps [36].

To investigate the influence of 1,25-(OH)₂D₃ on epithelial cell-derived inflammation, we evaluated the expression of IL-33 in nasal tissues with immunofluorescence microscopy. IL-33 is the most recent addition to the IL-1 cytokine family [37]. IL-33 localizes in the nucleus under steady-state conditions and is rapidly released from damaged cells in response to infection, injury and inflammation [38]; epithelial cell-derived IL-33 can activate ILC2 whom settled in the submucosal to produce large amounts of IL-5, IL-13 and small amount of IL-4 [39]. As IL-5 is essential for eosinophil recruitment, IL-13 induces mucus hyperproduction [40], and IL-4 plays a critical role in Th2 cell differentiation, thus to potentiate type-2 immune responses in allergic diseases [37,41]. VD₃ has been summarized to be efficacious in reducing epithelial cell-derived inflammation in the upper and lower airway and down-regulate the fibroblast production of chemokine ligand 5 (a powerful eosinophil chemoattractant) [42]. However, the expression of IL-33 in nasal tissue has not been reported. In our study, we found that IL-33 was highly expressed not only in the nucleus of epithelial cells but also in chief cells and some intercalated duct cells. However, OVA challenge significantly decreased the number of IL-33-positive epithelial cells, and 1,25-(OH)₂D₃ treatment increased the

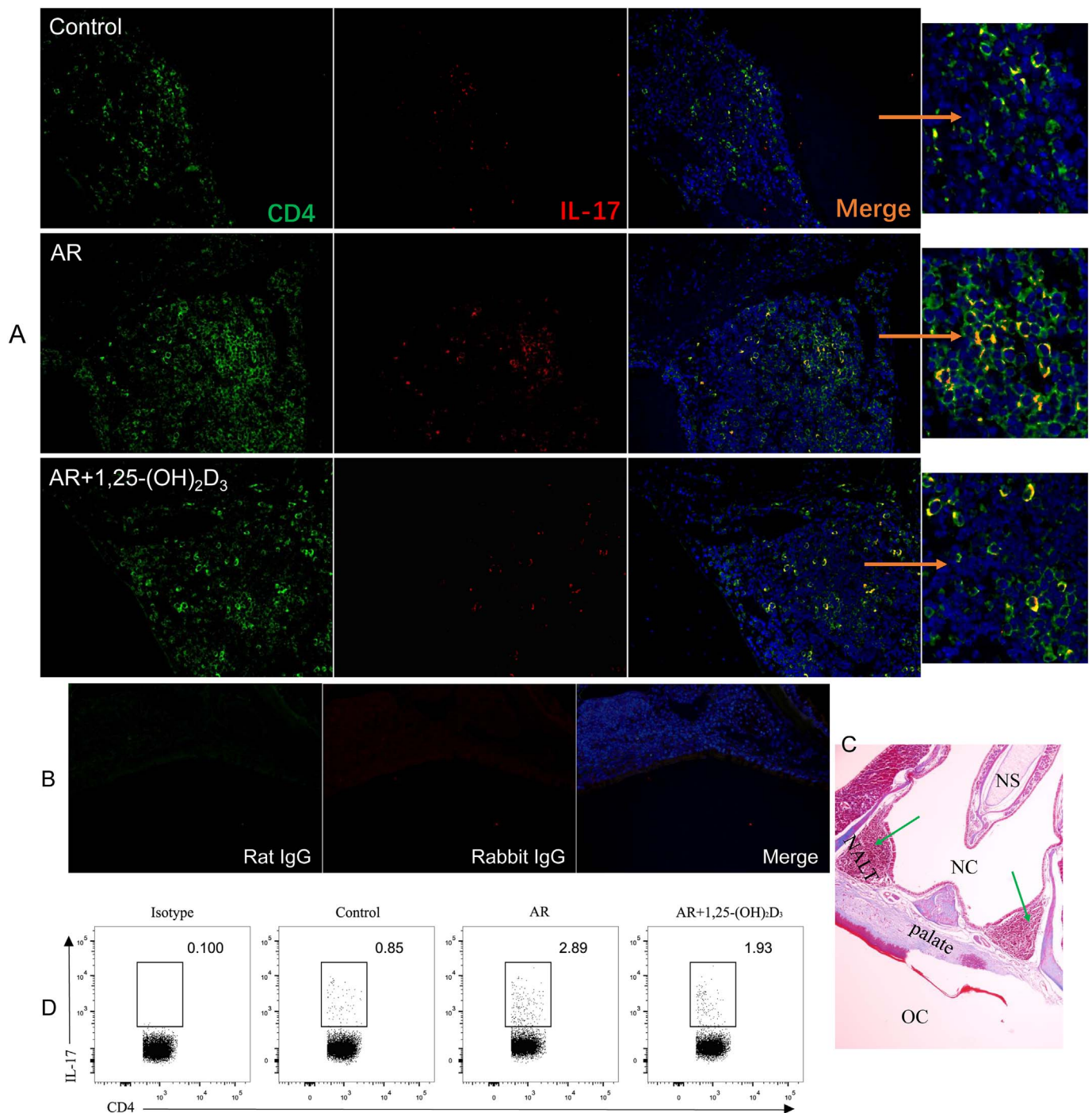


Fig. 5. (C) The location of NALT (Masson, ×40): NALT is located on the nasal surface of the palate. (A) Nasal sections (original magnification, ×400) were stained with rat anti-mouse CD4, rabbit anti-mouse IL-17, goat anti-rat-Alexa Fluor 488 (green), and goat anti-rabbit-Alexa Fluor 594 (red) antibodies, and nuclei were stained with DAPI (blue). (B) Rat IgG and rabbit IgG were used in control staining. CD4⁺ and IL-17⁺ double-positive cells were classified as Th17 cells. (D) Four to six NALT specimens were collected from each group, dissociated into single cells, and stained with anti-CD3-PE-CF594, anti-CD4-PerCP-Cy5.5 and anti-IL-17-Alexa Fluor 488 to analyze the percentage of Th17 cells by flow cytometry. OC, oral cavity, NC, nasal cavity, NS, nasal septum, NALT, nasal-associated lymphoid tissue, arrows point to NALT. This experiment had repeated twice and shown similar results. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

number of IL-33-positive epithelial cells in AR mice. 1,25-(OH)₂D₃ may function by alleviating local mucosal inflammation and maintaining more epithelial cells in a steady-state condition without releasing IL-33 from the nucleus. An *in vitro* study reported that human bronchial epithelial cells cultured with 1,25-(OH)₂D₃ exhibited increased expression of the soluble decoy receptor for IL-33 (sST2), which inhibits the actions of IL-33 [43]. Suggesting that 1,25-(OH)₂D₃ affect epithelial cell-derived IL-33-induced allergic responses by suppressing the release of IL-33 from the nucleus and increasing the expression of sST2.

Clinically, it has been demonstrated that VD3 supplementation benefits newly diagnosed asthma in children [44], and the inclusion of foods containing vitamin D in maternal diets during pregnancy can have beneficial effects during childhood [10]. As cigarette smoke has shown to inhibit VDR translocation on bronchial epithelial, suggesting that VD3 mainly play a role in the initial stage of the allergic airway diseases, and damaged tissues may limit its effectiveness, this may be the reason why the association between AR and VD3 is less consist in adults [45–47]. And the effect of VD3 on innate immunity more than

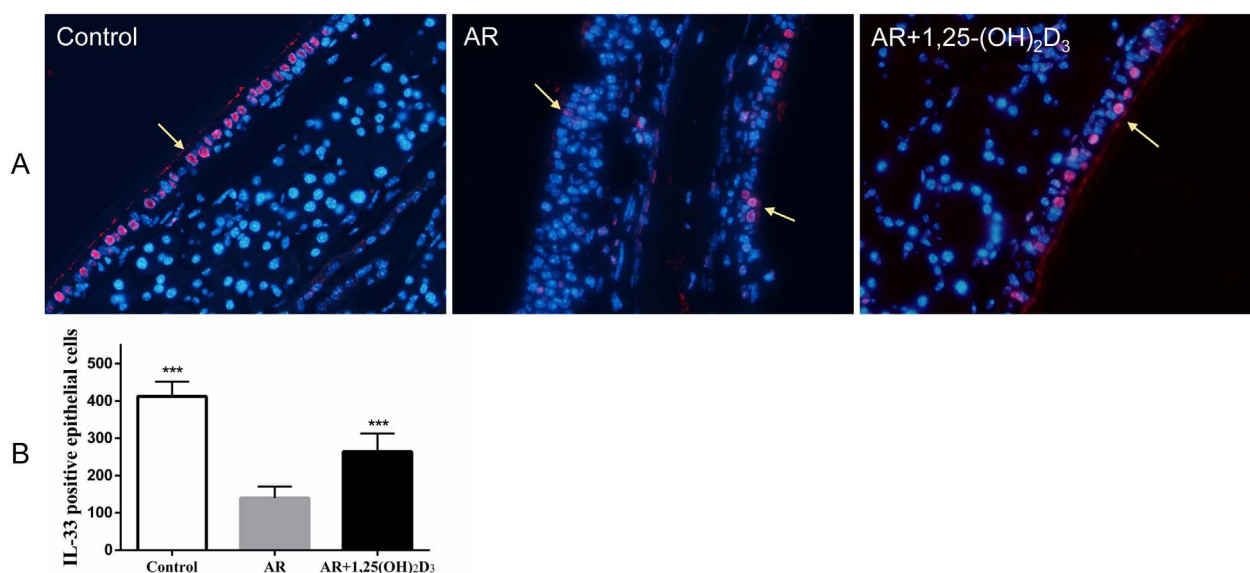


Fig. 6. (A) Epithelial cell nuclei are the main sources of IL-33 in nasal tissue. The number of IL-33-positive cells was significantly lower in OVA-induced AR mice, and treatment with 1,25-(OH)₂D₃ significantly increased the number of IL-33-positive cells in AR mice. Nasal sections (original magnification, × 400) were stained with rabbit anti-mouse IL-33 and goat anti-rabbit-Alexa Fluor 594 (red) antibodies, and nuclei were stained with DAPI (blue). IL-33 expression was viewed with a fluorescence microscope. (B) The number of IL-33-positive epithelial cells was counted in two sections from each mouse and six mice per group. Values represent the mean ± SD, $n = 6$. *** $P < 0.001$ vs. the AR group. Arrows point to epithelial cells. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

adaptive immunity researches are needed in this field.

In summary, our results suggest that 1,25-(OH)₂D₃ is beneficial in an AR model and that it functions by inhibiting local and systemic Th17 responses, suppressing the production of IL-5 and IL-13 cytokines in NALF and maintaining IL-33 under steady-state in nasal tissues.

Acknowledgments

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